**Report for Sean Schumacher**

**Differential analysis of PICRUSt2 results**

**February 2024**

**Methods**

Functional abundances were predicted using Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt2). Differential abundance (DA) analyses were conducted and visualized using the R package ggpicrust2 (Yang *et al*. 2023) using DESeq2 (Love *et al*. 2014). Significant level was set to adjusted p-value of 0.05.

Note: The analysis can be run with the following methods in addition to DESeq2: ALDEx2 (Fernandes *et al.* 2013), Maaslin2 (Mallick *et al.* 2021), LinDA (Zhou *et al.* 2022), edgeR (Robinson *et al.* 2010), limma voom (Ritchie *et al.* 2015), metagenomeSeq (Paulson *et al.* 2013), and lefser (Segata *et al.* 2011).

**Results**

All results are in the directory “Pathway\_DE\_analysis”. Labeling of each file consists of the test name, the company, the treatment, and the time comparison (Ex. “DESeq2\_Jackson\_EAE\_D0\_D21\_”). For comparisons between companies, the labeling consists of the test name, the treatment, and the time in days (Ex. “DESeq2\_Naive\_D21\_”).

Each comparison has the following files if there were significant pathways:

| **File Name** | **Description** |
| --- | --- |
| \*\*\_Heatmap\_plot.pdf | Heatmap using standard scores (Z-scores) for each DA test. |
| \*\*\_PCA\_plot.pdf | PCA plot for samples for each comparison |
| \*\*\_Error\_plot.pdf  | Relative abundance difference between groups, log2 fold change, and *P*-values derived from the top 15 significant DA results for each comparison.*Note: this can be changed to include more or fewer or specific pathways.*  |
| \*\*\_Top60\_Significant\_Pathway.csv | Top 60 significant DA scores (based on an adjusted p-value of 0.05) for each comparison |

**References**

Love, M.I., Huber, W., Anders, S. (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology*, 15:550. 10.1186/s13059-014-0550-8

Yang C, Mai J, Cao X, Burberry A, Cominelli F, Zhang L, Elofsson A. ggpicrust2: an R package for PICRUSt2 predicted functional profile analysis and visualization. Bioinformatics. 2023;[39](https://doi.org/10.1093/bioinformatics/btad470)([8](https://doi.org/10.1093/bioinformatics/btad470)):39. doi:10.1093/bioinformatics/btad470.